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Plantinga, TH; van der Does, C; Tomkiewicz, D; van Keulen, G; Konings, WN; Driessen, AJM; Plantinga, Titia H.

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Deletion of the *yiaMNO* transporter genes affects the growth characteristics of *Escherichia coli* K-12

Titia H. Plantinga,[1]† Chris van der Does,[1]‡ Danuta Tomkiewicz,[1] Geertje van Keulen,[2]§ Wil N. Konings[1] and Arnold J. M. Driessen[1]

Department of Molecular Microbiology[1] and Microbial Physiology[2], Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, PO Box 14, 9750, AA Haren, The Netherlands

Correspondence
Arnold J. M. Driessen
a.j.m.driessen@rug.nl

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Binding-protein-dependent secondary transporters make up a unique transport protein family. They use a solute-binding protein in proton-motive-force-driven transport. Only a few systems have been functionally analysed. The *yiaMNO* genes of *Escherichia coli* K-12 encode one family member that transports the rare pentose L-xylulose. Its physiological role is unknown, since wild-type *E. coli* K-12 does not utilize L-xylulose as sole carbon source. Deletion of the *yiaMNO* genes in *E. coli* K-12 strain MC4100 resulted in remarkable changes in the transition from exponential growth to the stationary phase, high-salt survival and biofilm formation.

INTRODUCTION

Prokaryotes employ several classes of transport systems for the uptake of solutes from their environment, which are defined on the basis of their subunit composition and mode of energization (Driessen *et al.*, 2000). The recently discovered binding-protein-dependent secondary (Driessen *et al.*, 1997, 2000), or tripartite ATP-independent periplasmic (TRAP) (Forward *et al.*, 1997; Rabus *et al.*, 1999; Kelly & Thomas, 2001) transporters utilize a solute-binding protein that captures the substrate at the outside of the cell and deliver it to a membrane permease that is made up of two subunits. The large subunit contains 12 putative transmembrane domains (TMDs) and a large cytoplasmic loop between TMD 6 and TMD 7, and thus resembles classical secondary transporters. The small subunit is made up of four putative TMDs. Transport is driven by the proton-motive force (pmf), in contrast to the more familiar binding-protein-dependent ATP-binding cassette (ABC) transporters that are energized by ATP hydrolysis (Driessen *et al.*, 1997, 2000; Forward *et al.*, 1997; Rabus *et al.*, 1999; Kelly & Thomas, 2001; Wyborn *et al.*, 2001).

To date, only a few members of this class of transporters have been functionally described. One member is involved in the sodium- and pmf-dependent uptake of glutamate by *Rhodobacter sphaeroides* (Jacobs *et al.*, 1996). The DctPQM transporter of *Rhodobacter capsulatis* transports the C_4-dicarboxylates malate, succinate and fumarate (Forward *et al.*, 1997) and a similar system was found in *Wolinella succinogenes* (Ullmann *et al.*, 2000). More recently, TeaABC of *Halomonas elongata* was shown to transport the compatible solutes ectoine and hydroxyectoine (Grammann *et al.*, 2002). Consequently, these transporters may play roles both in the uptake of carbon sources and in the protection of the cell against non-favourable conditions. The *Escherichia coli* K-12 genome contains one binding-protein-dependent secondary transporter encoded by the *yiaMNO* genes, located in the *yiaKLMNOPQRS* gene cluster. *yiaO* encodes the periplasmic binding protein, while *yiaM* and *yiaN* encode the small and large membrane protein, respectively. Based on identity with the genes encoding TeaABC, YiaMNO has been suggested to be involved in the uptake of osmoprotectants (Ly *et al.*, 2004). However, experimental evidence indicates that YiaMNO catalyses the uptake of the rare pentose L-xylulose (L-*threo*-2-pentulose) (Plantinga *et al.*, 2004a). L-Xylulose is presumably metabolized by the enzymes encoded immediately downstream of the *yiaMNO* genes, since L-xylulose transport and metabolism is found only in cells that constitutively express the *yiaK-S* gene cluster. This coupling of the proposed carbohydrate transport and metabolism function is conserved in at least 26 bacterial genomes, most of which are human pathogens (Plantinga *et al.*, 2004b). However, L-xylulose does not induce expression of these genes and little is known about its utilization by *E. coli* K-12 (Ibañez *et al.*, 2000; Plantinga *et al.*, 2004a), indicating that L-xylulose might not be the sole substrate for the YiaMNO transporter.

This study focuses on the physiological role of the *yiaMNO* genes. We have characterized *E. coli* strain K-12 MC4100, which is widely used in gene expression work (Peters *et al.*,

†Present address: Max-Planck-Institute for Infection Biology, Schumannstraße 21–22, D-10117 Berlin, Germany.

‡Present address: Institute of Biochemistry, Biocenter, Goethe-Universität Frankfurt, Marie Curie Straße 9, D-60439 Frankfurt, Germany.

§Present address: Department of Molecular Microbiology, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK.

2003), and a Δ*yiaMNO* derivative thereof. Some striking phenotypical differences between the Δ*yiaMNO* mutant and parent strain were observed in our growth experiments.

METHODS

Bacterial strains and growth conditions. All strains that were used in this study are *E. coli* K-12 derivatives: MC4100 [F^- *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flbB5301 deoC1 ptsF25 rbsR*; Casabadan, 1976]; ECL1 [HfrC *phoA8 relA1 tonA22* $T2^r$ (*λ*); Lin, 1976]; JA134 (ECL1 *lyx*$^+$; Sánchez *et al.*, 1994); MG1655 (F^- *λ*$^-$ *ilvG rfb50 rph1*; Blattner *et al.*, 1997). The unmarked chromosomal deletion of the *yiaMNO* genes was created in strains MC4100, JA134 and MG1655 as described elsewhere (Plantinga *et al.*, 2004a) and the respective mutants were labelled TP001, TP018 and TP010. Cells were grown aerobically in Luria–Bertani (LB), LB supplemented with 0·5 % (w/v) glucose (LBG) or M63 minimal medium (Miller, 1972) at 37 °C. Growth was monitored by measurements of OD_{660}. The number of c.f.u. was determined by plating serial dilutions on LB-agar. Antibiotics were used at 50 μg ml^{-1} and 12 μg ml^{-1} for ampicillin and tetracyclin, respectively. For high-salt growth experiments, LB and LBG were supplemented with 0·7–1·0 M NaCl or KCl.

Expression of the *yiaM* and *yiaO* genes. Cells were grown aerobically in LB and LBG, samples were taken at various OD_{660} values and total RNA was extracted as described by Plantinga *et al.* (2004a). RT-PCR was performed on 1 μg total RNA using RT-PCR beads (Amersham Pharmacia Biotech) and primers directed against 474 and 632 bp fragments of the *yiaM* and *secY* genes, respectively (Plantinga *et al.*, 2004a). For Northern Blot analysis, 5 μg total RNA from each sample was run on a 1·2 % (w/v) agarose gel containing 6·7 % (v/v) formaldehyde and transferred to a positively charged nylon membrane (Zeta Probe; Bio-Rad) in 150 mM NaCl, 15 mM sodium citrate. For preparation of the probe, the *yiaO* gene was cloned via PCR using forward primer 5′-AATGGATCCATT-AAAAGGAAAATATTATG-3′ and reverse primer 5′-CCCTCTA-GATTATTGCACCTCATCCAC-3′, introducing *Bam*HI and *Xba*I restriction sites, respectively. The fragment was ligated into vector pET401 (Van der Sluis *et al.*, 2002) for propagation and subsequently used as a template for labelling with [^{32}P]dCTP using Klenow polymerase (Roche). The probe was purified using a PCR product isolation procedure (Qiagen) and hybridized overnight in 0·5 M sodium phosphate, pH 7·2, 1 % (w/v) blocking reagent (Roche) and 7 % (w/v) SDS at 65 °C. The membrane was washed in 50 mM sodium phosphate, pH 7·2, containing 1 % SDS, and the signal was recorded by autoradiography.

Promoter induction and *β*-galactosidase assays. The 1000 bp upstream region of *yiaM* was cloned via PCR. The forward primer (5′-ATGGTGGATCCGATGATGAGGGCA-3′) introduced a *Bam*HI site and the reverse primer (5′-TGAATTCATAGCTATTCCTT-GAGGC-3′) introduced an *Eco*RI site. The fragment was translationally fused to a *lacZ*-reporter gene in vector pBC3 (Meijer *et al.*, 1991). This vector, termed pP1000, was transformed into strain MC4100. Heat and cold shock were applied by shifting liquid cultures to 42 and 10 °C, respectively. High salt, sucrose and spent medium (prepared as described below) effects were determined by harvesting the cells and resuspending them in the respective media. Induction by L-xylulose was tested by harvesting the cells and resuspending them in minimal medium containing 0·4 % L-xylulose. Following 30 min of incubation, the cells were harvested and *β*-galactosidase assays were performed as described by Miller (1992). Values were expressed in Miller units (MU).

Spent medium growth experiments. MC4100 and TP001 cells were grown aerobically in LB or LBG, 50 ml samples were taken at OD_{660} values of 0·5, 1·5, 2·0 and 2·5, and harvested (4000 ***g***, 10 min, 4 °C). The supernatant was filtered to remove all cells. Fresh LB or LBG cultures of MC4100 and TP001 were grown to an OD_{660} of 0·5, harvested and resuspended in equal volumes of pre-warmed (37 °C) spent medium. Aerobic growth was continued and monitored over time.

NMR analysis. MC4100 and TP001 were grown to an OD_{660} of 0·5 in a total of 8 l LBG containing 0·9 M NaCl per strain. Cells were harvested and freeze-dried overnight. Ethanol extracts for determination of intracellular solutes were prepared as described elsewhere (Martins & Santos, 1995). ^{13}C-NMR spectra were measured by Dr A. Ramos at the Instituto Technologia Química e Biológica of the University of Lisbon, Portugal, using a Bruker DRX500 spectrometer as described by Martins & Santos (1995).

Biofilms. Cells were plated on LB-agar and grown overnight at 37 °C. Colonies were picked and resuspended in M63 minimal medium and diluted into untreated polystyrene 96-wells plates (Costar) containing M63 0·5 % (w/v) glucose. Plates were incubated at 37 °C for 60 h and biofilm formation was quantified using crystal violet, as described by O'Toole *et al.* (1999).

RESULTS

Expression of the *yiaMNO* genes in *E. coli* strain MC4100

To determine the physiological role of the *yiaMNO* genes, we first studied their expression by RT-PCR. *E. coli* strain MC4100 was grown aerobically in LB or in LB supplemented with 0·5 % (w/v) glucose (Fig. 1a). Under these conditions the cells expressed the *yiaM* gene during growth in both LB and LBG (Fig. 1b, c). Expression appeared to be growth-phase-dependent and was maximal in the late stationary phase (Fig. 1b, c). Expression was not repressed in the presence of glucose (Fig. 1c). These results were supported by Northern Blotting using the *yiaO* gene as the hybridization probe, detecting a fragment of 8·2 kb, which is the expected size for the *yiaK-S* messenger (data not shown; Ibañez *et al.*, 2000).

E. coli strain JA134 expresses the *yiaK-S* gene cluster from one promoter upstream of the *yiaK* gene (Ibañez *et al.*, 2000). Computational analysis of this region on the *E. coli* K-12 genome, using RegulonDB, identified an additional putative promoter immediately upstream of *yiaM* (Salgado *et al.*, 2001). This putative promoter was translationally fused to a *lacZ*-reporter construct and its activity in strain MC4100 was determined. The detected expression pattern confirmed the RT-PCR and Northern Blotting results described above (data not shown), indicating that the *yiaM-S* promoter was active. However, this putative promoter was not induced by L-xylulose, as is the case for the *yiaK* promoter (Ibañez *et al.*, 2000), or by any of the conditions tested below (data not shown).

Deletion of the *yiaMNO* genes affects growth

MC4100 and its *yiaMNO* derivative TP001 were previously used in a search for the substrate of the YiaMNO transporter

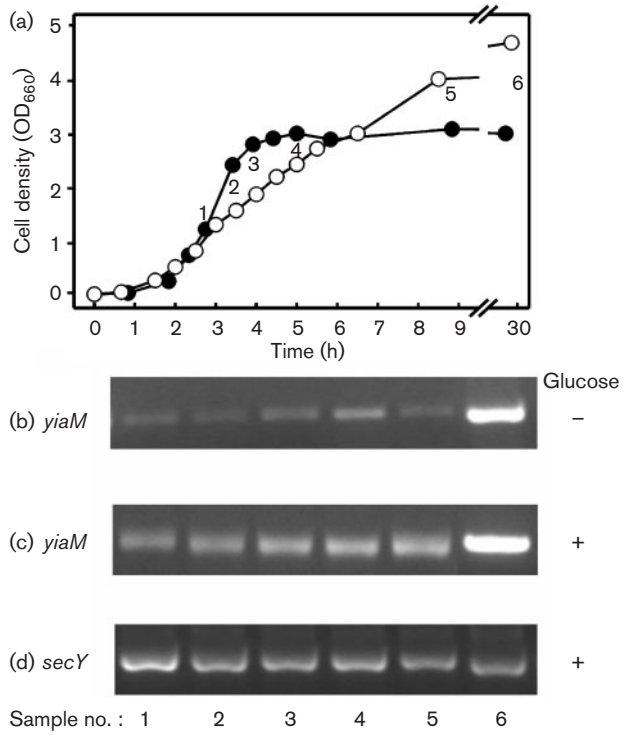

Fig. 1. Expression of the *yiaM* gene in MC4100 at different growth stages. (a) MC4100 cells were grown aerobically in LB (open circles) and LBG (closed circles). Total RNA was isolated at indicated growth phases: 1, exponential; 2–4, early (2), mid (3) and late (4) transition; 5, early stationary phase; 6, 24 h stationary phase. (b, c) RT-PCR demonstrates expression of the *yiaM* gene during these growth phases in LB (b) and in LBG (c). (d) *secY*, control for the constitutive expression of a membrane protein. Growth-phase-dependent expression of *yiaM* is observed, which is highest during the stationary phase.

(Plantinga *et al.*, 2004a). A striking phenotypical difference between mutant and parent strain was observed in growth experiments. When grown aerobically in LBG batch culture, i.e. containing glucose, both strains grew at nearly identical rates during the exponential phase, with the relatively sharp transition from exponential to stationary phase typical for a carbon-limited batch culture (Mason & Egli, 1993). However, for TP001 this transition was delayed, reproducibly yielding a higher final OD_{660} at the stationary phase (Fig. 2). This was supported by c.f.u. numbers (data not shown). This difference in growth was not observed in LB (Fig. 2). When the cells were growing in minimal media supplied either with glucose or other carbon sources, a similar difference in final OD_{660} was observed (data not shown; compounds listed in Plantinga *et al.*, 2004a). These findings suggest that, under specific conditions, the deletion of the three structural genes encoding the YiaMNO transporter affects growth, and in particular the ability of the cells to enter stationary phase.

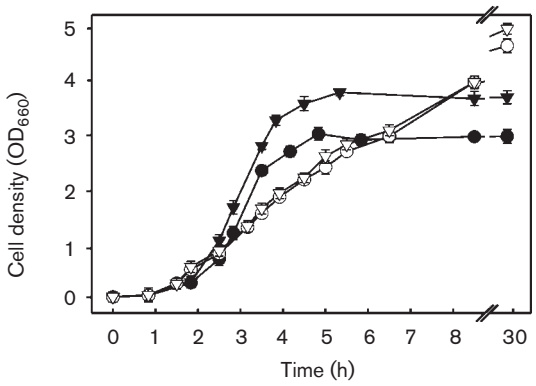

Fig. 2. Effect of the *yiaMNO* deletion on aerobic growth of MC4100 in rich medium. When grown in LBG (filled symbols), strain TP001 (triangles) reproducibly reached a higher final OD_{660} than MC4100 (circles), whereas no such difference was observed during growth on LB (open symbols).

Involvement of the YiaMNO transporter in quorum sensing

Interestingly, autoinducer 2 (AI-2), the quorum sensing signal molecule of *E. coli* and a range of other bacteria, is produced under conditions where the effects of the *yiaMNO*-deletion were observed (Surette & Bassler, 1998; Bassler, 2002; Chen *et al.*, 2002). Deletion of the AI-2-producing enzyme affects growth in a similar fashion (Sperandio *et al.*, 1999). Therefore, we investigated the involvement of the transporter, or the transported substrate(s), in quorum sensing by examining the effect of spent medium on growth. Spent medium taken from MC4100 cultures grown in LB hardly influenced the growth of fresh cultures (Fig. 3, black and white bars). In contrast, addition of LBG spent medium taken at an $OD_{660} > 1{\cdot}5$ clearly negatively affected growth (Fig. 3, dark grey and light grey bars). However, no differences in response were observed between MC4100 (Fig. 3, black and dark grey bars) and TP001 (Fig. 3, white and light grey bars). Spent medium prepared from TP001 yielded identical results (data not shown). These findings indicate that if the autoinducer is indeed produced by these strains during growth on LBG, the ability of TP001 to respond to the molecule is not affected by the deletion of the *yiaMNO* genes. We therefore conclude that YiaMNO is not involved in the uptake of AI-2 or any other compound with a similar function.

Deletion of the *yiaMNO* genes affects high-salt tolerance

The *H. elongata* TeaABC transporter protects the cell against hyperosmotic conditions (Grammann *et al.*, 2002) and it has been suggested that the YiaMNO transporter may play a similar role in *E. coli* (Ly *et al.*, 2004). Therefore, the ability of strains MC4100 and TP001 to survive hyperosmotic stress was investigated. Following dilution into LBG containing 0·9 M NaCl, which is 10-times the concentration of

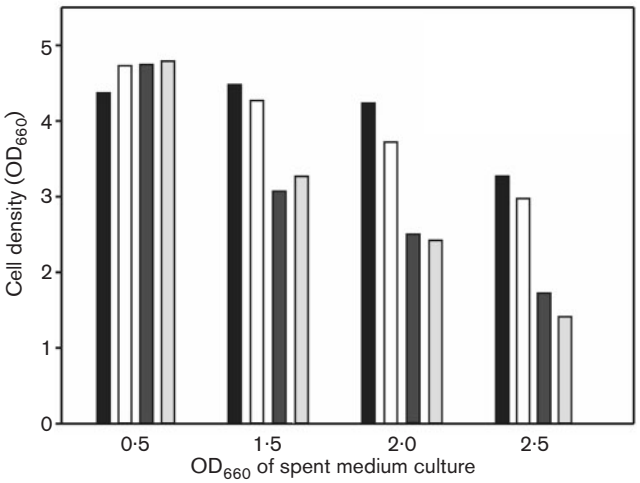

Fig. 3. Effect of MC4100 spent medium on growth. Cell-free MC4100 culture supernatants ('spent medium') were prepared from cultures grown on either LB or LBG to an OD_{660} of 0·5, 1·5, 2·0 and 2·5, respectively. Fresh cultures of MC4100 and TP001 were grown in LB to an OD_{660} of 0·5, harvested and resuspended as follows: black bars, MC4100 in LB spent medium; white bars, TP001 in LB spent medium; dark grey bars, MC4100 in LBG spent medium; light grey bars, TP001 in LBG spent medium. The final OD_{660} values after 7 h of incubation in spent medium are indicated. The data shown are representative for the experiment. An autoinducer-like activity is produced by MC4100 in LBG, decreasing the observed final OD_{660}, but both strains respond in an identical manner to its addition.

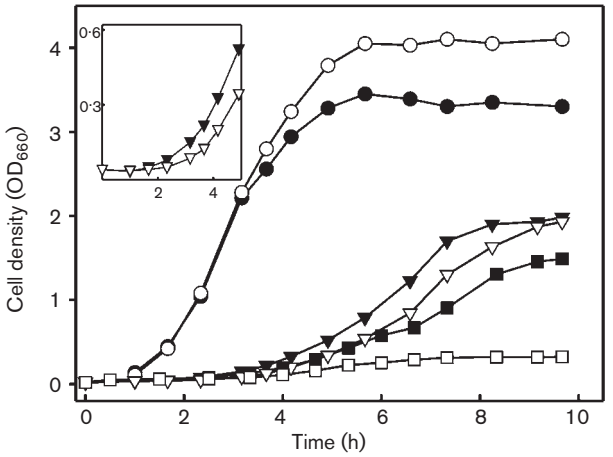

Fig. 4. High salt sensitivity of the *yiaMNO* deletion mutant TP001. Both parent MC4100 (filled symbols) and mutant TP001 (open symbols) were grown in LBG containing 100 mM (normal concn, circles), 0·9 M (triangles and inset) or 1 M (squares) NaCl. A representative experiment is shown. The ability of TP001 to respond to high-salt stress decreased with increasing salt concentration.

NaCl in LB(G), MC4100 started doubling after a lag time of 1 h 48 min ± 8 min (six independent experiments) and reached a final OD_{660} of about 2 (Fig. 4, black triangles). However, growth of the deletion mutant was delayed at this high salt concentration, starting an additional 1 h 22 min ± 12 min ($n=6$) after the wild-type strain had resumed growth (Fig. 4, inset). TP001 did reach the same final OD_{660} of about 2 (Fig. 4, white triangles). Strain TP001 did not grow at all at 1 M NaCl (Fig. 4). Similar results were obtained when the cells were grown in the presence of high KCl concentrations, but not in media containing high sucrose (data not shown). This suggests that the YiaMNO transporter may indeed be involved in the accumulation of an osmoprotective compound. To identify a possible substrate involved in this process, we performed ^{13}C-NMR analysis of whole-cell extracts obtained from both *E. coli* strains grown to an OD_{660} of 0·5 in LBG containing 0·9 M NaCl. However, no differences in total accumulated cellular compounds could be detected (data not shown). The major compatible solute that had been accumulated by both strains was identified as glycine betaine. Growth of both MC4100 and TP001 on M63 minimal medium with glucose as sole carbon source was also negatively affected by NaCl at concentrations above 0·7 M. Again, TP001 showed a delay in recovery from the high-salt challenge (data not shown). In these experiments, insufficient biomass was obtained to allow for ^{13}C-NMR analysis of whole-cell extracts. However, growth of both strains could be restored to identical levels by the addition of 1 mM of the osmoprotectants glycine betaine, L-proline, potassium glutamate or ectoine (data not shown). In this regard, none of these compatible solutes are substrates for the YiaMNO transporter (Plantinga *et al.*, 2004a).

Deletion of the *yiaMNO* genes reduces biofilm formation

Loss of a 2·4 kb genomic fragment containing the *yiaMNO* homologues of the Gram-negative bacterium *Ralstonia* sp. TFD14 (Nakatsu *et al.*, 1998) led to changes in bile salt sensitivity and adhesion (Riley *et al.*, 2001). Since the size of the complete deletion was not determined, it remains unknown whether the YiaMNO homologue contributes to this phenotype. Therefore, we investigated whether the ability to attach to surfaces is affected in the *yiaMNO* mutant of *E. coli*. Biofilms were allowed to form for 60 h in M63 minimal medium in the presence of D-glucose as sole carbon source. As observed before, TP001 reached a higher final OD_{660} compared to MC4100 during growth (data not shown). However, biofilm formation was clearly negatively affected by deletion of the YiaMNO transporter (Fig. 5). This demonstrates that changes in adhesion may indeed be attributed to deletion of the *yiaMNO* genes.

DISCUSSION

An increasing number of whole-genome expression profiles has been published in recent years (e.g. Tao *et al.*, 1999; Arfin

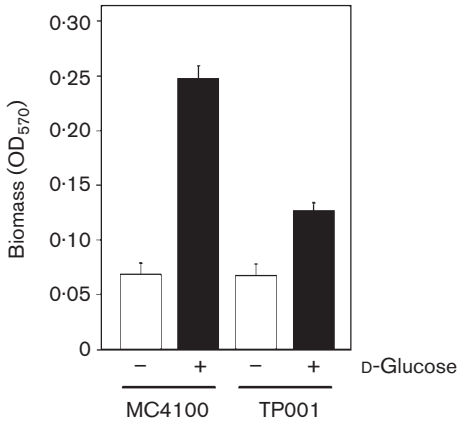

Fig. 5. Biofilm formation by MC4100 and TP001 detected by crystal violet staining of surface-attached biomass. Cells were grown for 60 h in M63 minimal medium in the absence (–) or presence (+) of D-glucose. In the presence of D-glucose both strains form biofilms, but this is reduced in the *yiaMNO* deletion mutant in comparison to the wild-type strain.

et al., 2000; Arnold *et al.*, 2001; Pomposiello *et al.*, 2001; Wei *et al.*, 2001; Beloin *et al.*, 2004; Ren *et al.*, 2004; Kang *et al.*, 2005). Although a range of physiological conditions have been tested in these studies with various *E. coli* K-12 strains, including the sequenced strain MG1655, no specific pattern of expression of the *yiaK-S* genes was detected. Expression of the *yiaK-S* gene cluster has been observed in *E. coli* strain JA134, a derivative of K-12 strain ECL1, following IS*1*-mediated disruption of the *yiaJ* gene immediately upstream of *yiaK* (Badía *et al.*, 1998). The disrupted *yiaJ* gene encodes the repressor of the gene cluster and the event leads to constitutive expression of the *yiaK-S* genes in strain JA134 (Badía *et al.*, 1998). Activation of this gene cluster had previously been shown to lead to the expression of an L-xylulose kinase and allowed strain JA134 to grow on L-lyxose (Sánchez *et al.*, 1994). However, L-lyxose is neither an inducer of *yiaK-S* expression (Ibañez *et al.*, 2000) nor is it a substrate for the transporter encoded by the *yiaMNO* genes, which has recently been shown to transport the rare pentose L-xylulose (Plantinga *et al.*, 2004a). As strain JA134 lacks the YiaJ repressor of the *yiaK-S* genes, we cannot exclude the possibility that different regulatory circuits are active in this strain. Thus, the combined available data does not shed light on the physiological role of these genes. The study presented here addresses the physiological function of the *yiaMNO* genes, encoding a binding-protein-dependent secondary transporter. We made use of *E. coli* K-12 MC4100, a strain widely used in gene expression studies, which expresses the genes in a growth-phase-dependent manner (Fig. 1).

Although expression of the *yiaK-S* gene cluster in strain JA134 is subject to carbon catabolite repression via the cyclic AMP receptor protein (CRP) (Ibañez *et al.*, 2000), in strain MC4100 expression does not appear to be repressed by glucose (Fig. 1). Interestingly, transcription of the *yiaK* promoter is highly upregulated in the mouse pathogen *Salmonella enterica* serovar Typhimurium SL1344 during colonization of the caecum of a murine enteritis model, and to a lesser extent in a murine typhoid fever model (C. Rollenhagen and D. Bumann, personal communication). Moreover, the promoter is also active in an *in vitro* model mimicking the conditions in the gut, i.e. low oxygen and increased NaCl concentration (C. Rollenhagen and D. Bumann, personal communication). These recent findings therefore support the notion that the YiaMNO transporter may play a role in scavenging scarce substrates under the limiting conditions encountered by this pathogen inside a eukaryotic host. Indeed, in *E. coli* strain MC4100, we detect the highest expression levels in cells that had been in stationary phase for 24 h (Fig. 1). Since L-xylulose does not induce expression of the YiaMNO transporter, while expression does not appear to be repressed by glucose, a major role of this system in carbon source uptake and utilization is not evident at this time.

The loss of the YiaMNO transporter clearly affects growth of strain MC4100, in particular the transition from exponential to stationary growth on LBG (Fig. 2). In the *yiaMNO* mutant this transition was delayed, yielding a higher final OD_{660} during stationary phase. Strikingly, this may be related to a phenomenon observed in *Ralstonia* sp. TFD14. After 1000 generations of experimental evolution, all evolved populations showed an increased fitness compared to the ancestor strain (Korona *et al.*, 1994) and 71 out of 72 evolved populations had lost the 2·4 kb genomic fragment containing the *Ralstonia yiaMNO*-homologues (Nakatsu *et al.*, 1998). The size of the complete deletion was not determined, but our data now clearly demonstrate that this growth advantage may be attributed to the *yiaMNO* deletion. The observed increased fitness may be related to a defective quorum sensing circuit, in which the transporter is involved in the uptake of a signalling molecule that negatively affects growth. However, although our data suggest that strain MC4100 produces an 'autoinducer-like' activity, the YiaMNO transporter is not required for transport of the autoinducer or a precursor thereof (Fig. 3).

In the evolved populations of *Ralstonia* sp. the extracellular polysaccharide (EPS) had disappeared, leading to changes in bile salt sensitivity and adhesion properties (Riley *et al.*, 2001). Although the *yiaMNO* mutant is clearly more sensitive to high salt, we were unable to identify a possible transported substrate involved in osmoprotection. The major EPS of *E. coli* K-12, colanic acid (Rick & Silver, 1996), is required for development of biofilm architecture (Prigent-Combaret *et al.*, 1999; Danese *et al.*, 2000). It has been shown that deletion of the producing genes delays, but does not abolish, biofilm formation, with nearly identical amounts of surface-attached biomass at times $\geqslant$45 h (Danese *et al.*, 2000). However, we still observe major differences in biofilm formation after 60 h (Fig. 5), therefore colanic acid biosynthesis most likely is not affected by the *yiaMNO* deletion.

Nevertheless, the possible (indirect) involvement of the YiaMNO transporter in EPS biosynthesis does provide a basis for future experiments.

The *yiaMNO* derivatives of two additional strains were used in the biofilm experiments to test whether the observed effects are strain-specific. No effect of the deletion on biofilm formation was found in strain JA134, which, as its parent ECL1, formed significantly higher amounts of biofilm than strain MC4100 in the assay (data not shown). The effect of the deletion may therefore have been obscured in this genetic background (see above). Deletion of the *yiaMNO* transporter in the sequenced K-12 strain MG1655, yielding strain TP010, did however reduce biofilm formation (data not shown). In this regard, a negative effect similar to that observed in strain TP001 was observed during growth of strain TP010 on LB(G) in the presence of high salt (data not shown). Although significant genomic differences have been detected between both strains (Peters *et al.*, 2003) these do not localize to the region encoding the *yiaMNO* genes and our findings do not appear to be strain-specific.

Concluding remarks

The phenotypic effects we have observed in the *yiaMNO* mutant of *E. coli* K-12 strain MC4100 are diverse and the pathways that underlie these phenomena are complex. However, the observations made with *Ralstonia* sp. support our finding that a localized deletion may have drastic effects on growth of the organism. In particular, the possible role of the transporter and its transported substrate in EPS formation deserves further experimental investigation. In addition, the recent findings in *Salmonella* support the assumption that the YiaMNO transporter functions under limiting substrate conditions, where L-xylulose is an intermediate in eukaryotic metabolism. The co-localization of carbohydrate transport and metabolism functions is found in a range of pathogenic bacteria (Plantinga *et al.*, 2004b), suggesting these systems may play important roles in survival during infection. Further studies are required to establish the physiological role of the binding-protein-dependent secondary transporter encoded by the *E. coli* K-12 *yiaMNO* genes.

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REFERENCES

Arfin, S. M., Long, A. D., Ito, E. T., Tolleri, L., Riehle, M. M., Paegle, E. S. & Hatfield, G. W. (2000). Global gene expression profiling in *Escherichia coli* K-12. The effects of integration host factor. *J Biol Chem* **275**, 29672–29684.

Arnold, C. N., McElhanon, J., Lee, A., Leonhart, R. & Siegele, D. A. (2001). Global analysis of *Escherichia coli* gene expression during the acetate-induced acid tolerance response. *J Bacteriol* **183**, 2178–2186.

Badía, J., Ibañez, E., Sabaté, M., Baldomà, L. & Aguilar, J. (1998). A rare 920-kilobase chromosomal inversion mediated by IS*1* transposition causes constitutive expression of the *yiaK-S* operon for carbohydrate utilization in *Escherichia coli*. *J Biol Chem* **273**, 8376–8381.

Bassler, B. L. (2002). Small talk: cell-to-cell communication in bacteria. *Cell* **109**, 421–424.

Beloin, C., Valle, J., Latour-Lamber, P. & 8 other authors (2004). Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. *Mol Microbiol* **51**, 659–674.

Blattner, F. R., Plunkett, G., III, Bloch, C. A. & 14 other authors (1997). The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453–1462.

Casabadan, M. J. (1976). Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J Mol Biol* **104**, 541–555.

Chen, X., Schauder, S., Potier, N., Van Dorsselaer, A., Pelczer, I., Bassler, B. L. & Hughson, F. M. (2002). Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* **415**, 545–549.

Danese, P. N., Pratt, L. A. & Kolter, R. (2000). Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. *J Bacteriol* **182**, 3593–3596.

Driessen, A. J. M., Jacobs, M. H. J. & Konings, W. N. (1997). A new family of prokaryotic transport proteins: binding protein-dependent secondary transporters. *Mol Microbiol* **24**, 879–883.

Driessen, A. J. M., Rosen, B. P. & Konings, W. N. (2000). Diversity of transport mechanisms: common structural principles. *Trends Biochem Sci* **25**, 397–401.

Forward, J. A., Behrendt, M. C., Wyborn, N. R., Cross, R. & Kelly, D. J. (1997). TRAP transporters: a new family of periplasmic solute transport systems encoded by the *dctPQM* genes of *Rhodobacter capsulatus* and by homologs in diverse Gram-negative bacteria. *J Bacteriol* **179**, 5482–5493.

Grammann, K., Volke, A. & Kunte, H. J. (2002). A new type of osmoregulated solute transporter identified in halophilic members of the *Bacteria* domain: TRAP-transporter TeaABC mediates uptake of ectoine and hydroxyectoine in *Halomonas elongata* DSM 2581^{T}. *J Bacteriol* **184**, 3078–3085.

Ibañez, E., Campos, E., Baldomà, L., Aguilar, J. & Badía, J. (2000). Regulation of expression of the *yiaKLMNOPQRS* operon for carbohydrate utilization in *Escherichia coli*: involvement of the main transcriptional factors. *J Bacteriol* **182**, 4617–4624.

Jacobs, M. H. J., Van der Heide, T., Driessen, A. J. M. & Konings, W. N. (1996). Glutamate transport in *Rhodobacter sphaeroides* is mediated by a novel binding protein-dependent secondary transport system. *Proc Natl Acad Sci U S A* **93**, 12786–12790.

Kang, Y., Weber, K. D., Qiu, Y., Kiley, P. J. & Blattner, F. R. (2005). Genome-wide expression analysis indicates that FNR of *Escherichia coli* K-12 regulates a large number of genes of unknown function. *J Bacteriol* **187**, 1135–1160.

Kelly, D. J. & Thomas, G. H. (2001). The tripartite ATP-independent periplasmic (TRAP) transporters of bacteria and archaea. *FEMS Microbiol Rev* **25**, 405–424.

Korona, R., Nakatsu, C. H., Forney, L. J. & Lenski, R. E. (1994). Evidence for multiple adaptive peaks from populations of bacteria evolving in a structured habitat. *Proc Natl Acad Sci U S A* **91**, 9037–9041.

Lin, E. C. C. (1976). Glycerol dissimilation and its regulation in bacteria. *Annu Rev Microbiol* **30**, 535–578.

Ly, A., Henderson, J., Lu, A., Culham, D. E. & Wood, J. M. (2004). Osmoregulatory systems of *Escherichia coli*: identification of Betaine-Carnitine-Choline Transporter family member BetU and distributions of *betU* and *trkG* among pathogenic and nonpathogenic isolates. *J Bacteriol* **186**, 296–306.

Martins, L. O. & Santos, H. (1995). Accumulation of mannosylglycerate and di-*myo*-inositol-phosphate by *Pyrococcus furiosus* in response to salinity and temperature. *Appl Environ Microbiol* **61**, 3299–3303.

Mason, C. A. & Egli, T. (1993). Dynamics of microbial growth in the decelerating and stationary phase of batch culture. In *Starvation in Bacteria*, pp. 81–102. Edited by S. Kjelleberg. New York: Plenum.

Meijer, W. G., Arnberg, A. C., Enequist, H. G., Terpstra, P., Lidstrom, M. E. & Dijkhuizen, L. (1991). Identification and organization of carbon dioxide fixation genes in *Xanthobacter flavus* H4-14. *Mol Gen Genet* **225**, 320–330.

Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Miller, J. H. (1992). *A Short Course in Bacterial Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Nakatsu, C. H., Korona, R., Lenski, R. E., De Bruijn, F. J., Marsh, T. L. & Forney, L. J. (1998). Parallel and divergent genotypic evolution in experimental populations of *Ralstonia* sp. *J Bacteriol* **180**, 4325–4331.

O'Toole, G. A., Pratt, L. A., Watnick, P. A., Newman, D. K., Weaver, V. B. & Kolter, R. (1999). Genetic approaches to study of biofilms. *Methods Enzymol* **310**, 91–109.

Peters, J. E., Thate, T. E. & Craig, N. L. (2003). Definition of the *Escherichia coli* MC4100 genome by use of a DNA array. *J Bacteriol* **185**, 2017–2021.

Plantinga, T. H., Van der Does, C., Badia, J., Aguilar, J., Konings, W. N. & Driessen, A. J. M. (2004a). Functional characterization of the *Escherichia coli* K-12 *yiaMNO* transport protein genes. *Mol Membr Biol* **21**, 51–57.

Plantinga, T. H., Van der Does, C. & Driessen, A. J. M. (2004b). Transporter's evolution and carbohydrate metabolic clusters. *Trends Microbiol* **12**, 4–7.

Pomposiello, P. J., Bennik, M. H. J. & Demple, B. (2001). Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. *J Bacteriol* **183**, 3890–3902.

Prigent-Combaret, C., Vidal, O., Dorel, C. & Lejeune, P. (1999). Abiotic surface sensing and biofilm-dependent regulation of gene expression in *Escherichia coli*. *J Bacteriol* **181**, 5993–6002.

Rabus, R., Jack, D. L., Kelly, D. J. & Saier, M. H. (1999). TRAP transporters: an ancient family of extracytoplasmic solute-receptor-dependent secondary active transporters. *Microbiology* **145**, 3431–3445.

Ren, D., Bedzyk, L. A., Thomas, S. N., Ye, R. W. & Wood, T. K. (2004). Gene expression in *Escherichia coli* biofilms. *Appl Microbiol Biotechnol* **64**, 515–524.

Rick, P. D. & Silver, R. P. (1996). Enterobacterial common antigen and capsular polysaccharides. In *Escherichia coli and Salmonella: Cellular and Molecular Biology*, pp. 104–122. Edited by F. C. Neidhardt. Washington, DC: American Society for Microbiology.

Riley, M. S., Cooper, V. S., Lenski, R. E., Forney, L. J. & Marsch, T. L. (2001). Rapid phenotypic change and diversification of a soil bacterium during 1000 generations of experimental evolution. *Microbiology* **147**, 995–1006.

Salgado, H., Santos-Zavaleta, A., Gama-Castro, S., Millan-Zarate, D., Diaz-Peredo, E., Sanchez-Solano, F., Perez-Rueda, E. & Collado-Vides, J. (2001). RegulonDB (version 3.2): transcriptional regulation and operon organization in *Escherichia coli* K-12. *Nucleic Acids Res* **29**, 72–74.

Sánchez, J. C., Gímenez, R., Schneider, A., Fessner, W.-D., Baldomà, L., Aguilar, J. & Badia, J. (1994). Activation of a cryptic gene encoding a kinase for L-xylulose opens a new pathway for the utilization of L-lyxose by *Escherichia coli*. *J Biol Chem* **269**, 29665–29669.

Sperandio, V., Mellies, J. L., Nguyen, W., Shin, S. & Kaper, J. B. (1999). Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci U S A* **96**, 15196–15201.

Surette, M. G. & Bassler, B. L. (1998). Quorum sensing in *Escherichia coli* and *Salmonella typhimurium*. *Proc Natl Acad Sci U S A* **95**, 7046–7050.

Tao, H., Bausch, C., Richmond, C., Blattner, F. R. & Conway, T. (1999). Functional genomics: expression analysis of *Escherichia coli* growing on minimal and rich media. *J Bacteriol* **181**, 6425–6440.

Ullmann, R., Gross, R., Simon, J., Unden, G. & Kröger, A. (2000). Transport of C_4-dicarboxylates in *Wolinella succinogenes*. *J Bacteriol* **182**, 5757–5764.

Van der Sluis, E. O., Nouwen, N. & Driessen, A. J. M. (2002). SecY-SecY and SecY-SecG contacts revealed by site-specific crosslinking. *FEBS Lett* **527**, 159–165.

Wei, Y., Lee, J.-M., Richmond, C., Blattner, F. R., Rafalski, J. A. & LaRossa, R. A. (2001). High-density microarray-mediated gene expression profiling of *Escherichia coli*. *J Bacteriol* **183**, 545–556.

Wyborn, N. R., Alderson, J., Andrews, S. C. & Kelly, D. J. (2001). Topological analysis of DctQ, the small integral membrane protein of the C4-dicarboxylate TRAP transporter of *Rhodobacter capsulatus*. *FEMS Microbiol Lett* **194**, 13–17.